Differentiating Two Forms of Plasma Lipase by Use of Media with Different Ionic Strengths

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Three different techniques of lipase (EC 3.1.1.3) determination (titrimetry, nephelometry, and enzyme immunoassay) were used to investigate an interesting effect of ionic strength on enzyme activity. Both activation in the presence of NaCl (140 mmol/L) and strong immuno-inhibition were observed for lipase from plasma of subjects with acute pancreatitis. Another type of lipase, not associated with pancreatitis and only weakly immuno-inhibited, showed maximum activity in the presence of 80 mmol/L NaCl. The pancreatitis-type lipase was activated by 140 mmol/L NaCl solution only if colipase and sodium deoxycholate were also present, which suggests that these components are cofactors in the activating effect and confirms the specificity of this property. These findings can be exploited to improve the diagnosis of acute pancreatitis.

Additional Keyphrases: isoenzymes • pancreatitis • immuno-inhibition • colipase • deoxycholate

Diagnosis and monitoring of acute pancreatitis depend strongly on biochemical tests such as those for amylase and lipase. Development of tests for lipase has continued for two reasons: (a) the recent introduction of turbidimetric or nephelometric methods (1–3), often considered to be more convenient than the older titrimetric techniques (4, 5), especially if colipase is used (3), and (b) the greater information supplied by determination of lipase in acute pancreatitis (6, 7).

We recently reported (8) that added NaCl, 140 mmol/L, increased the extent of the lipase–triolein reaction, as measured by nephelometry, in the presence of colipase and biliary salts.

We then wanted to verify whether the addition of NaCl increased the rate of release of fatty acids when lipase was added to a triolein emulsion. The second goal of the present work was to better characterize this effect of ionic strength and to evaluate whether it would have value in the diagnosis of acute pancreatitis.

Materials and Methods

Studied populations. Population 1 consisted of 50 plasma samples received for lipase determination and otherwise unselected. Their lipase activity as well as the NaCl effect was determined without delay—although storage for two months at −20 °C has no effect on lipase activity or degree of activation.

Population 2 consisted of 61 plasma samples (from hospitalized adults), which were stored at −20 °C and used for studies of correlation between results by nephelometry (with and without added NaCl) and immunoenzyme assay.

Population 3 consisted of 36 plasma samples from hospitalized adults with high lipase activity. Of these samples, 32 were from patients with well-defined acute pancreatitis; the remaining four samples were from patients without symptoms of acute pancreatitis (wide infra). All these samples were used for the study of the activation profile, and some of them for immuno-inhibition.

Nephelometer. The nephelometer was built in-house (9). It has a laser source (λ = 632.8 nm), photodiodes positioned at 50°, 70°, and 90° from the transmitted beam, and a thermostatting (37 °C) compartment with a Peltier heating element. The signal is measured in millivolts, as previously described (8, 9). In the presence of colipase and sodium deoxycholate the reaction was zero order under the conditions we used.

Reagents. Commercially available porcine lipase (EC 3.1.1.3) was used (Boehringer-Mannheim Corp., Mannheim, F.R.G.). Porcine colipase, prepared as previously described (10), contained no lipase activity. Sodium deoxycholate was purchased from E. Merck, Darmstadt, F.R.G. An olive-oil emulsion was prepared as previously described (5). For comparison, we also used a commercially available reagent for lipase determination (UV system; Boehringer-Mannheim).

Titrimetry. We used the Tietz–Fiereck procedure (5), with slight modifications: To 3 mL of olive oil emulsion, add 0.50 mL of distilled water and 0.66 mL of Tris buffer (0.2 mol/L, pH 9.2). After mixing and incubation at 37 °C, add 0.30 mL of sample. Stop the reaction 75 min later by adding 1 mL of absolute ethanol. Titrate with 50 mmol/L NaOH solution, and express lipase activity as the number of milliliters of 50 mmol/L NaOH required to adjust the pH to 10.5. Measure the apparent activity of a blank (distilled water instead of enzyme) run with each sample.

Nephelometry. This was essentially as described elsewhere (8). Lipase activity (U/L) was expressed as micromoles of fatty acids released per minute per liter.

Enzyme immunoassay. Incubate 0.2 mL of diluted standard or sample for 2 h at room temperature in plastic tubes coated with sheep antibodies to lipase ("Enzygnost" lipase; Hoechst-Behring Corp., Marburg, F.R.G.). After washing with a phosphate buffer (66 mmol/L, pH 7.2) containing Tween 20 [polyoxyethylene (20) sorbitan monolaurate], add 0.2 mL of rabbit anti-lipase labeled with peroxidase; incubate for 2 h at room temperature. After a second washing with the phosphate buffer/Tween 20, add the substrate (o-phenylenediamine/hydrogen peroxide). After 30 min at room temperature, stop the reaction by adding 1 mL of 0.25 mol/L sulfuric acid. Measure the absorbance of the mixture at 492 nm.

Immuino-inhibition. Incubate 0.2 mL of diluted sample for 2 h at room temperature in tubes coated with sheep antibodies to lipase (Hoechst-Behring Corp.), then nephelometrically determine the lipase activity in 50 μL of the incubated sample. Compare the results with the lipase activity of the same diluted sample incubated for 2 h at 37 °C in uncoated plastic tubes (no antibodies).
Results

Titrmetric assessment of nephelometric measurements. To verify that addition of NaCl actually activated the lipase rather than simply altered the micellar structure nonenzymically so as to change the light scatter, we used titrmetry. Not only did the addition of colipase and sodium deoxycholate increase the rate of release of oleic acid, but also (Figure 1) the activity of lipase, whether determined by titrmetry or nephelometry, was maximum in the presence of 140 mmol of NaCl per liter (for porcine pancreatic lipase).

Mean lipase activation in plasma. We studied the lipase activating effect of 140 mmol/L NaCl for plasma samples from 50 adults (population 1). Lipase activity was increased to various degrees, ranging from 0.70- to 4.9-fold (51–1625 U/L). For subjects whose lipase activity in plasma was less than 160 U/L (the upper limit of the normal reference interval), the mean (± SD) increase was 1.54 (± 0.62) fold. For the remaining subjects (n = 35), whose lipase activity exceeded 160 U/L, the mean increase was 2.88 (± 0.74) fold, significantly greater (p <0.001 by Student’s t-test). Given that the separation into two groups was based only on the measured lipase activity (less or greater than 160 U/L), this result at first suggested that the effect of NaCl might depend on the amount of enzyme in the sample. To test this, we studied the activation effect with aqueous dilutions of three different samples:

(a) purified porcine pancreas enzyme,
(b) pooled plasma from subjects suffering from acute pancreatitis, and
(c) pooled plasma from healthy subjects.

As Figure 2 shows, the extent of the NaCl effect did not depend on the dilution but rather on the kind of sample used. No increase of lipase activity was observed at 140 mmol of NaCl per liter when plasma from healthy subjects was used, whereas porcine enzyme as well as plasma lipase from diseased subjects increased their activity by threefold.

Activity vs NaCl concentration. Further to investigate these variations in the degree of activation, we measured by nephelometry the effect of different concentrations of NaCl on lipase activity of various samples (Figure 3). Such a “profile” for each sample was compared with that for the purified porcine pancreas lipase. For healthy subjects, lipase activity peaked in the presence of 80 mmol of NaCl per liter. Plasma from a patient with increased lipase activity (960 U/L) but without clinical symptoms of pancreatitis showed similar results.

On the other hand, lipase from patients with clinically confirmed acute pancreatitis showed two peaks of activity, one at 80 mmol of NaCl per liter, the other at 140 mmol/L. The first peak probably corresponds to a lipase that is present in both healthy and diseased subjects; the other may correspond to a lipase released in the plasma during acute...
pancreatitis. Figure 3 shows profiles for two such patients. Results were similar for serum or plasma from 30 more subjects (population 3) with clinical symptoms of acute pancreatitis (not shown).

Comparison between nephelometry and an enzymic immunoassay. We compared the results by our nephelometric method with those by an enzyme immunoassay (Hoechst-Behring) involving the use of sheep and rabbit antibodies to lipase. This "sandwich"-type assay is considered to be more specific for human pancreatic lipase (11). The correlation between the two methods is strong for 61 plasma samples (population 2) containing lipase activity ranging from 28 to 1227 U/L (y = 1.31x + 126 and r = 0.841, where y = U/L as determined by nephelometry without NaCl, and x = μg of pancreatic lipase per liter as measured by the enzyme immunoassay). However, including NaCl (140 mmol/L final concentration) in our nephelometric procedure improved the correlation with the enzyme immunoassay and decreased the y-intercept (y = 1.07x + 54; r = 0.873).

Pancreatic lipase immunoinhibition. We tried to inhibit pancreatic lipase activity in samples corresponding to a well-defined clinical state. We incubated plasma samples (diluted 10-fold in water) for 2 h in plastic tubes coated with anti-lipase antibodies, then measured lipase activity by nephelometry. All samples from patients with acute pancreatitis showed maximum lipase activity in the presence of 140 mmol/L NaCl and more than 60% of their initial lipase activity was immunoinhibited (Table 1). In contrast, the last four samples listed—which were from subjects with high lipase activity, low plasma amylase, no clinical symptoms of pancreatitis, and, moreover, exhibited a maximum lipase activity at 80 mmol/L NaCl—were inhibited by anti-lipase antibodies by less than 31%.

Discussion

The addition of NaCl increases the rate of release of fatty acids by porcine-pancreas lipase. The same effect is also observed with other salts, such as CH₂COONa or KCl; thus this is evidently an effect of ionic strength. The activation also requires the presence of both colipase and biliary salts (8).

Borgström et al. (12) also stated that the binding of colipase to triglycerides was dependent on salt concentration; at 150 mmol/L NaCl, colipase can bind to triglyceride substrate even in "supramolecular" concentrations of bile salt, whereas NaCl as great as 1 mol/L did not influence the binding between lipase and colipase. On the other hand, we have observed that sodium deoxycholate at supramolecular concentrations can stimulate the lipase–triglyceride reaction only if colipase and NaCl, 140 mmol/L, are present (not shown). Our findings concerning the dependence of the ionic strength effect on the presence of both colipase and sodium deoxycholate are thus consistent with the data of Borgström et al.

Although the exact mechanism of the effect of ionic strength on pancreatic lipase activity is not fully elucidated by these results, they are consistent with the presence of multiple forms of lipase activity in some plasmas. We believe at least two different forms of lipase are present in the plasma of subjects suffering from acute pancreatitis. The first one has maximum activity in the presence of 80 mmol of NaCl per liter (under our assay conditions) and is also present in plasma of healthy subjects. The second form exhibits maximum activity in the presence of 140 mmol of NaCl per liter and appears to be present in subjects with clinical symptoms of acute pancreatitis.

Vogel and Zieve have previously reported (13) that serum of patients with acute pancreatitis contains a lipase with maximum activity at pH 9.1 and sodium deoxycholate concentration of 8.5 mmol/L, whereas lipase activity in normal serum or duodenal contents had different pH and bile salt requirements. Their study included an olive-oil emulsion but no colipase.

Desnuelle (14) reported two distinct fractions of lipase activity after purification of a pancreatic extract on a Sephadex G200 column, one of which (the "fast" lipase) was probably a complex of the enzyme with phospholipid-rich micelles.

Our findings lead to a similar conclusion but with an additional criterion. Given (a) the strong correlation between results by our nephelometric technique (in the presence of 140 mmol/L NaCl) and those by the immunoenzymatic test, (b) the considerable inhibition of lipase by antibodies to pancreatic lipase in samples from subjects with acute pancreatitis, and (c) the increased specificity of colipase (3) when NaCl and sodium deoxycholate are added to the medium at the concentrations described here, we conclude that this interesting property of pancreatic lipase should be taken into account in improving the diagnostic efficiency of this test for acute pancreatitis. In particular, if 140 mmol of NaCl per liter is included in the medium, the activity observed for samples from patients without acute pancreatitis will be lower; their activation degree would be less than 1.5, because the usual pancreatic enzyme will be activated threefold. Ideally, the profile of activation by NaCl (at 0, 80, and 140 mmol/L) should be evaluated in all ambiguous cases.

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References


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Table 1. Lipase Activity In 10 Plasma Samples after Treatment With Anti-Lipase Antibodies

<table>
<thead>
<tr>
<th>Lipase (by nephelometry), U/L</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Inhibition, %</th>
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<tr>
<td>938</td>
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The first six samples listed here were from six patients with acute pancreatitis; lipase activity was maximum in the presence of 140 mmol of NaCl per liter. The last four samples had maximum lipase activity with 80 mmol of NaCl per liter, and all four patentes were characterized by renal insufficiency, intact pancreas (by echotomography), and normal values for plasma amylase.

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